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Treatment Strategies for Human Arboviral Infections Applicable to Veterinary Medicine

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INTRODUCTION

Over 40 arboviruses are known to infect domestic animals. Some of the viruses cause only subclinical infections as indicated by the presence of antibodies. Overt infections in domestic animals can be classified according to three syndromes caused by these viruses: systemic febrile illness, hemorraghic fever, or encephalitis.[1] The distinction by these criteria is not definite because agents that cause encephalitis or hemorrhagic fever can also cause systemic febrile illness or even subclinical infections, as in the case of Rift Valley fever virus (RVFV).

Clinical and pathological syndromes of systemic infections vary widely with causative agents including fetal malformations, abortions, lesions at multiple locations, edema, and cardiac failures affecting horse, cattle, goat, sheep, and swine. Encephalitis occurs mostly in horses, whereas hemorraghic diseases occur in sheep, swine, deer, and cattle. The viruses responsible for the infections in animals also cause human disease with different severity from that in animals. The most important arboviruses causing disease in human and domestic animals are included in TABLE 1. The mode of transmission of the viral agents is by infected arthropods like mosquitos, ticks, sandflies, and midges. The transmission cycle can involve a single vertebrate host and a single vector, or a complex cycle with different hosts and vectors.

Arboviral infections in domesticated animals can be controlled by surveillance, immunization, vector control, and chemotherapy (TABLE 2). Surveillance is based on reporting cases of clinical illness and on the presence of serum antibodies. Surveillance is not always attainable because of (1) remote wild reservoirs and vectors, (2) a need for a network of diagnostic laboratories and trained personnel, and (3) cost. Surveillance has been applied successfully, as in cases of St. Louis encephalitis and yellow fever virus. Immunization provided protection against several arboviruses, but several drawbacks eclipse its practicality. The existence of multiple serotypes does not provide full cross-protection; single administration of the vaccine is seldom sufficient for long-term protection; and other general problems exist such as reversion of an attenuated strain, resources, and availability. Vector control by spraying is not effective in large forests, but it has been applied successfully to reduce vector densities in a well-defined limited area. Control of domesticated animals by antivirals is a new, explorable approach.

Preventive control by antiviral substance can be considered only when it has a relatively broad spectrum of activity; otherwise, identification of the causative agent renders it impractical. In contrast to a conventional antiviral that inhibits a particular

TABLE 1. Overt Infections Caused by Arboviruses in Humans and Domestic Animals

Family/Virus	Animal[a]	Disease	Human Disease
Togavirus			
Eastern equine encephalitis	E	Encephalitis	Same
Getah	E	Febrile systemic, diphasic rash	
Semliki Forest	E	Encephalitis	Same
Venezuelan equine encephalitis	E	Febrile systemic, encephalitis	Same
Western equine encephalitis	E	Encephalitis	Same
Flaviridae			
Japanese encephalitis	P	Abortions	Encephalitis
	E	Subclinical, encephalitis	
Louping ill	S	Febrile systemic, encephalitis	Encephalitis
Wesselsbron	S	Hemorrhagic fever	Febrile systemic
Bunya Akabane	C, S, G	Congenital abnormalities	
Crimean-Congo hemorrhagic fever	C, S, G	Mild illness	Hemorrhagic fever
Nairobi sheep disease	S, G	Hemorrhagic, gastroenteritis	
Rift Valley fever	S, G, C	Bloody diarrhea, abortions	Febrile systemic, encephalitis
Reoviridae			
African horse sickness	E	Febrile, pulmonary, cardiac, illness	None
Bluetongue	S	Hemorrhages, edemas, lameness	None
Rhabdoviridae			
Bovine ephemeral fever	C	Febrile systemic, respiratory	None
Vesicular stomatitis	C, E, S, P	Vesicles in the mouth and on feet	None
Iridoviridae			
African swine fever	P	Diarrhea, pneumonia, hemorrhages	None

[a] *Abbreviations:* E, equine; P, pig; S, sheep; C, cattle; G, goat.

TABLE 2. Preventive Measures to Control Arboviral Infections in Domestic Animals

Surveillance	
	Costly and labor intensive
Immunization	
	During epizootic, only live vaccines are effective
Vector Control	
	Not always practical
Antivirals	
	To be explored

enzyme not universally present among all viruses, an immunomodulator can provide protection against a broad spectrum of viral infections by evoking the specific and nonspecific antiviral defenses of the host; therefore, the identity of the etiologic agent is not relevant for the commencement of the treatment regimen. Both the specific and the nonspecific antiviral immune responses are initiated by the macrophages via release of cytokines, which provide a triggering signal for a cascade of mediators and immunological events.

EXPERIMENTAL PROCEDURES

Since 1983, extensive intramural and extramural efforts have been devoted in our Institute to the evaluation of the antiviral potential of immunomodulatory substances.[2-12] According to their origin, these substances can be classified into four groups, although the classification is not always clear-cut (TABLE 3): (1) synthetic compounds or chemicals, (2) microbial or natural products, (3) regulatory, biological substances, and (4) thymic hormones. With the exception of thymic hormones, about 60 of the listed substances were evaluated in animal models against arboviruses and to a lesser extent against herpesviruses containing RNA or DNA, respectively. A large number (about 20%) of these substances have prophylactic and/or therapeutic efficacy against the test viruses (TABLE 4). With the exception of interferon (INF), among the substances regulating the biological responses only interleukin-2 had a marked prophylactic efficacy. Most of these biologicals were produced by recombinant technology, but a few natural isolates were evaluated as well.

Preliminary and advanced evaluations of candidate compounds are performed in small and large laboratory animals against viruses representing all four families of arboviruses that cause infections with the same or similar syndromes in humans. Efficacy is expressed with long-term survivors, resistance to reinfection, elimination of virus from blood and organs, presence of virus-neutralizing antibodies, induction of serum INF and its related 2′-5′ adenylate synthetase (AS), and stimulation of selected biological markers. Safety of administration is monitored by clinical chemistry and by determination of the safety margin index. Compounds selected for advanced development are evaluated in the same manner in nonhuman primates. Compounds selected for human use are subjected to pharmacology and toxicology studies in small and large animals. In humans, these compounds are tested for safety of administration as indicated by hematological and clinical chemistry evaluations. During the safety study, modulation of the immune response and its biological markers are assessed to predict efficacy in two clinical studies. Efficacy in humans is assessed by resistance to sandfly fever virus that causes mild, self-limiting febrile disease. Parameters in humans encom-

TABLE 3. Immunomodulatory Substances with Antiviral Potential

Synthetic Compounds or Chemicals	Microbial and Natural Products	Regulatory Substances	Thymic Hormones
Poly(ICLC)	*N. rubra* extract	INF-α	Thymic humoral factor
Poly(ICDX)	Trehalose dimycolate	INF-β	Thymostimulin
Ampligen	*S. typhimurium* extract	INF-γ	Thymosan F-5
MDP	*C. parvum* extract	IL-1	Thymosin α-1
MTP-PE	S. 209 extract	IL-2	Thymulin
Poly I:C	*C. burnetii* extract	IL-4	Pentapeptide (synthetic)
Acridine-HCI	Bestatin	IL-6	
Quinolinamine	Xerosin	Tumor necrosis factor	
ABPP	FK-565	Colony-stimulating factor	
ABMP	BCG	Lymphotoxins	
AIPP	*B. abortus* extract		
Avridin	Purified endotoxin		
OK-432	Pseudogen		
Nucleosides	*B. pertussis* extract		
MVE-2	Sea plant extracts		
Azimexon	Plant polysaccharide extracts		
Cimetidine			
Diethyldithiocarbonate (DTC)			
Isoprinosin			
Murabutyde			
Indomethacin			
Tuftsin			
AM-3			
Streptonegrin			
Mannozyme			
Levamisole			

TABLE 4. Immunomodulators with Advanced Developmental Potential

Compound	Source	Prophylactic[c]	Therapeutic[c]
POLY-(ICDX)	Roswell Park Memorial Institute	++++	++++
POLY-(ICLC)[a,b]	NIAID, NIH	++++	++++
POLY I:C_{12}U (Ampligen)[a]	Johns Hopkins University	+++	++++
Poly I:C-Microcapsules[a]	Southern Research Institute	++++	NT
CL-246738[a]	Lederle	++++	++++
AVS-1018[a]	Riker Labs - 3M	++++	++++
Human recombinant α-INT[a]	Hoffman-LaRoche, Ciba-Geigy	++++	++++
Mouse recombinant γ-INT[a,b]	Cetus	++++	++++
ABPP[a]	Upjohn	++++	++++
ABMP[a]	Upjohn	++++	NT
AIPP[a]	Upjohn	++++	++
MTP-PE[a,b]	Ciba-Geigy	+++	+++
C. burnetti extract[a]	USAMRIID	+++	+
Trehalose dimycolate[a,b]	Ribi	++++	NT
S. typhimurium extract[b]	Ribi	+++	NT
C. parvum extract[b]	Ribi	+++	NT
S. 209 (unknown extract)[b]	Ribi	++	NT
Plant polysaccharide extract[c]	Anver Bioscience	++++	++++
Interleukin-2[a]	CETUS	0	+++
Tumor necrosis factor[a,b]	Genentech	0	0
Interleukin-1 and -4	Dupont, Immunex	0	0
M-CSF	CETUS	0	0

[a] Arbovirus.
[b] Herpesvirus.
[c] 0 indicates not active (no survivors); + = Marginal efficacy, 25% survivors; ++ = Moderate efficacy, 26-50% survivors; +++ = Marked efficacy, 51-75% survivors; ++++ = High efficacy, 76-100% survivors; NT = Not tested.

pass reduction of viremia, antibody response, and stimulation of selected parameters of specific and nonspecific immune functions.

RESULTS

The compounds, which were evaluated extensively with preliminary and/or advanced evaluation for their prophylactic efficacy. are shown in TABLE 5. One or more representatives of each of the four arbovirus families and two strains of herpesviruses were used in rodents (mice or guinea pigs) or in nonhuman primates. The scoring system was based on percent survival time attained with each substance, as indicated in the footnote. In rodents, these infections caused 80-100% mortality. In nonhuman primates, the mortality depended on the strain of the monkey. In studies involving monkeys, when the mortality of the control was 30% or less, a circulating virus titer was used to measure efficacy, as indicated in the footnote. Poly(ICLC), ampligen, and CL-246738 had the best overall prophylactic efficacy. Poly(ICLC) is a double-stranded polyriboinosinic-polyribocytidilic acid (polyIC) stabilized with poly-l-lysine and carboxymethyl-cellulose. It was developed following recognition that poly(ICLC), although effective against viral infections in mice,[13] is rapidly hydrolyzed by high amounts of nuclease present in the serum of humans. Poly(ICLC) and CL246738, an acridine hydrochloride (AH), evoke complete protection against viral infections representing three out of four arbovirus families. Ampligen, a mismatched double-stranded analogue of polyIC, with every 12 cytidine base replaced by uridine, is as effective in mice on prophylactic administration as poly(ICLC). Ampligen was developed for short-term stimulation of INF and the INF-activated biological response. The mismatch makes the double-stranded RNA more accessible to endonuclease, thus reducing its half-life.[14] Ampligen is the opposite of poly(ICLC) with respect to resistance to endonuclease, but the level of this enzyme in the serum of mice is very low; therefore, it does not interfere with the activity of this substance in this host. In order to increase the safety margin of poly(ICLC), defined as the ratio between the dose that is toxic to 50% of the test animals (mice) and the smallest dose evoking maximum protection against the infection, carboxymethyl-cellulose was replaced with completely biodegradable dextran (Dx), which has been used as a plasma expander in humans. By this replacement, the safety margin index of poly(ICDx) was increased three- to fourfold, without any reduction of the prophylactic efficacy evoked against RVFV-induced infection. Poly(ICLDx) has not yet been evaluated against other viral infections.

Compound AVS 1018, a quinolinamine (QA) free base, administered in 2% lactic acid solution, evoked excellent protection against lethal viral infections representing Bunyavirus and Flavivirus families. QA compound evoked only marginal protection against alphavirus [Venezuelan equine encephalitis virus (VEE)] infection, but it was not evaluated against the entire panel of RNA and DNA viruses in our preclinical developmental process. Human recombinant hybrid interferon-alpha B/D (HuRINF-αB/D), produced by Ciba- Geigy, Basel, Switzerland, provided very effective protection against viruses of all three arbovirus families, using a dose of 10^4 International Units (IU) per treatment. In comparison to HuRINF-αB/D, recombinant mouse INF-γ was less effective against Bunya- and Flaviviruses; however, it was equally effective against alphaviridae. Among several substituted pyrimidinone derivatives, ABPP [5-bromo-2,3-dihydro-2-imino-6-phenyl-4(1H)-pyrinidinone] and AIPP [5-bromo-2,3-dihydro-2-imino-6-phenyl-4(1H)-pyrimidinone] were the most effective substances to control arboviral infections.[6] In comparison with poly(ICLC), AH or QA, on prophylactic administration ABPP and AIPP were only slightly less effective. Against herpes simplex

TABLE 5. Prophylactic Efficacy of Immunomodulators against Arboviral Infections in Rodents and Nonhuman Primates

Compound (Source)	Mouse								Guinea Pig	Monkey	
	Bunyaviridae			Flavivirus	Alphavirus		Miscellaneous		Arena Virus	Bunyaviridae	Flavivirus
	Rift Valley Fever	Punta Toro	Carapuru	Banzi	Semliki F.	Venezuelan Equine Enceph.	Herpes Simplex Pneumonitis	Herpes Simplex Enceph.	Pichinde	Rift Valley Fever	Yellow Fever
Poly (ICLC) (NIAD, NIH)	++++	++++	++++	++++	++++	++++	+++	+++++	0		+++++
Poly(ICDX) (Roswell Park, USAMRIID)	++++										
Ampligen (Johns Hopkins U.)	++	++++	+++	++++	++++	++++	+++	++++			
CL-246, 783 (Lederle)	++++	++++	+++	++++	++++		+++	++++			+++++*
AVS-1018 (Riker-3M)	++++			++++		+					++++
Human recombinant INF-alpha (Ciba-Geigy, Hoffman LaRoche)	+++		++++	++++	++	+++		++++		++++*	++++
Mouse recombinant INF-gamma (Genentech)			++	++	++++	++++		++++		++*	
ABPP (Upjohn)	++++	++++	+	+++	++++	++++	++++		0		
AIPP (Upjohn)	+	+++	+	+++	++++	+	++++		0		

Key to symbols: 0 indicates not active (no survivors); +, marginal efficacy (up to 25% survivors); ++, moderate efficacy (26-50% survivors); +++, marked efficacy (51-75% survivors); ++++, high efficacy (76-100% survivors); ++*, moderate reduction of circulating virus titer; ++++*, complete reduction of circulating virus titer.

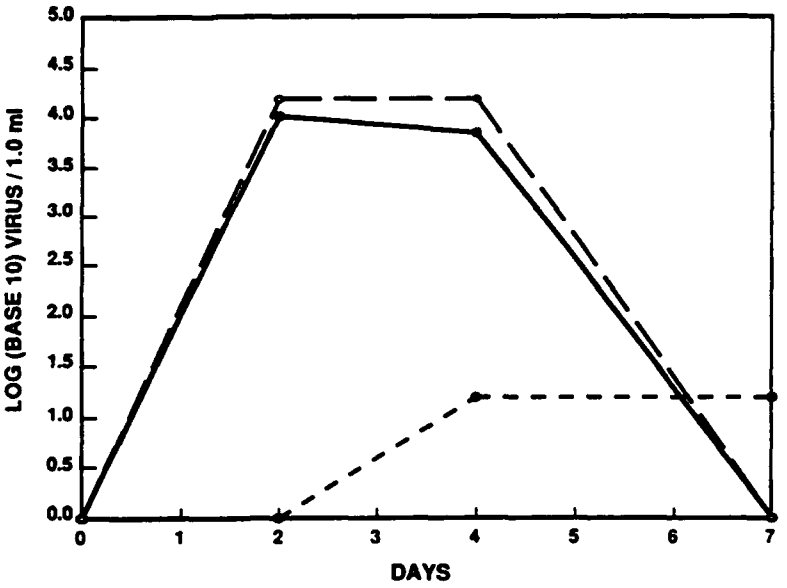

FIGURE 1. Suppression of yellow fever virus in the serum of squirrel monkeys treated with acridine-hydrochloride (AH). ●- - -● Monkeys ($n = 4$) were treated orally with 50 mg/kg AH every other day from day −1 to day 7 and were infected s.c. on day 0 with 10^5 PFU Asibi strain of yellow fever virus. ○- - -○ Monkeys ($n = 4$) were treated orally with 50 mg/kg AH every other day from days 1 to 7, and were infected s.c. on day 0 with 10^5 PFU Asibi strain of yellow fever virus. ○——○ Monkeys ($n = 4$) received placebo orally every other day from day −1 to day 7, and were infected s.c. on day 0 with 10^5 PFU Asibi strain of yellow fever virus.

virus they were as effective as the other top immunomodulators. ABPP, AIPP, AH, and QA are effective on oral administration.

None of the immunomodulators appeared to have any prophylactic efficacy against the Pichinde virus (a representative of arenaviruses, the fourth arbovirus family). Viruses of this family are known to be nonresponders to INF-α/β.

To predict efficacy in humans, four of the most effective immunomodulators were evaluated in nonhuman primates. A previous study at our Institute with poly(ICLC)[15] showed that when the treatment was initiated 8 h before or 8 h after infection, 70 and 75% of the respective rhesus monkeys were protected against lethal infection with the Asibi strain of yellow fever virus (TABLE 5).

The prophylactic potential of AH was evaluated in squirrel monkeys that have natural resistance to the neurotropic Asibi strain of yellow fever virus infection; therefore, the circulating virus titer was the main indicator of the antiviral reactivity of this compound (FIG. 1). Orally administered AH was given 24 h prior to yellow fever virus challenge. The compound evoked a marked antiviral activity by entirely reducing 3 logs of circulating virus titer. The compound was not effective when the treatment was initiated 24 h postinfection.

In cynomolgus monkeys when QA was administered (orally) beginning one day before infection with the Asibi strain of yellow fever virus and given every other day for 14 days, 8 logs of serum virus titer were completely reduced (FIG. 2). Although two of the three untreated infected monkeys died of yellow fever virus infection, all four treated monkeys survived the yellow fever virus challenge (TABLE 6).

Injection of 650 PFU Asibi strain of yellow fever virus to cynomolgus monkeys caused only low mortality, but high (> 5 logs) circulating virus titer was recovered

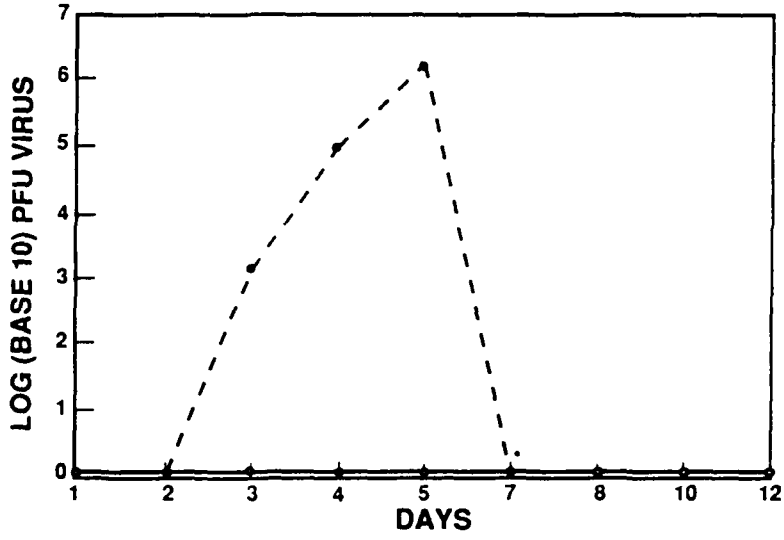

FIGURE 2. Yellow fever virus titer in the serum of cynomolgus monkeys treated with quinolinamine free base (QA). ○——○ Monkeys (n = 4) were treated orally with 20 mg/kg QA on day −1, and thereafter 10 mg/kg QA on every other day from day 0 to day 14. Challenge of 10^3 PFU Asibi strain of yellow fever was injected s.c. on day 0. ●——● Monkeys (n = 3) were treated orally with 2% Klucel on days −1, 0 and thereafter on every other day from day 1 to day 14. Challenge of 10^3 PFU Asibi strain of yellow fever virus was injected s.c. on day 0.

TABLE 6. Prophylactic Efficacy of Quinolinamine Free Base (AUS-1018) in Yellow Fever Virus-Infected Cynomolgus Monkeys

Group	Treatment	No. of Animals that Survived/Total
1	Virus[a] control	1/3
2	Virus[a] + quinolinamine[b]	4/4
3	Drug[c] control	2/2

[a] 10^3 PFU yellow fever virus injected i.m. on day 0.
[b] 20 mg/kg quinolinamine on day 1; 10 mg/kg on days 0, 2, 4, 6, 8, 10, 12, 14 orally in 2% Klucel.
[c] Treatment same as in group 2, without virus.

3-5 days postinfection (FIG. 3). Administration of 10^6 IU/kg of HuRINF-αB/D from one day before to seven days after infection reduced the virus titer almost entirely. Because of strong antiviral activity, the virus load was not sufficient to induce a viral-specific neutralizing antibody response.

Treatment of rhesus monkeys 24 h prior to RVFV infection with 5×10^5 units/kg of recombinant leukocyte A INF-α for 5 days, reduced the viremia with 5 logs (TABLE 5), limited hepatocellular damage and hemostatic derangement.[16] When five daily administrations of the same dose of this INF began 6 h after RVFV inoculation, the viremia was reduced by 2 logs, the viremic state was shortened considerably, and no clinical sign of the disease or laboratory evidence of impaired hemostasis was

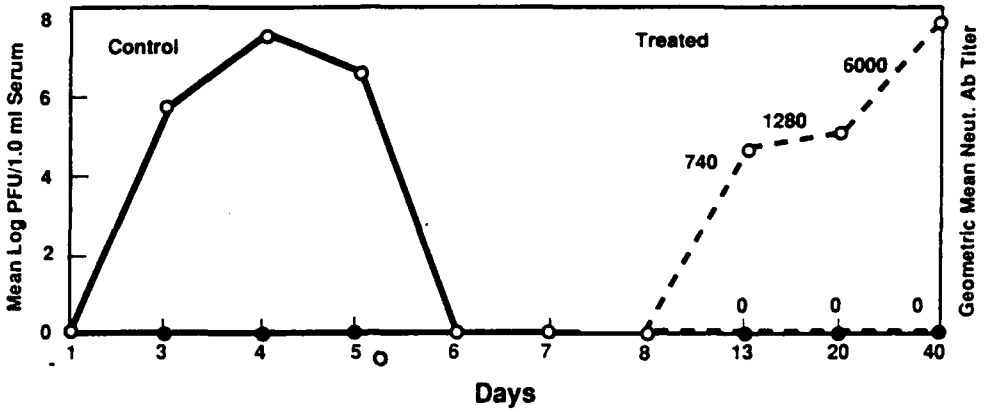

FIGURE 3. Yellow fever virus and neutralizing antibody titer in the serum of cynomolgus monkeys treated with human recombinant interferon-alphaB/D hybrid (HuRINF-αB/D). ●——● Monkeys (n = 4) were treated s.c. with 10^6 International Units of HuRINF-αB/D daily from day −1 to day 7, and were challenged s.c. on day 0 with 650 PFU Asibi strain of yellow fever virus. ○——○ Monkeys (n = 4) were treated s.c. with placebo daily from day −1 to day 7, and were challenged s.c. on day 0 with 650 PFU Asibi strain of yellow fever virus. ●- - -● Neutralizing antibody titer in HuRINF-αB/D treated monkeys. ○- - -○ Neutralizing antibody titer in placebo-treated monkeys. Neutralizing antibody titers were determined on Vero kidney fibroblast cells.

observed in comparison with the untreated controls. Prophylactic treatment of rhesus monkeys with 10^6 units/kg of recombinant human INF-γ given 24 h prior to RVFV infection and daily thereafter for a total of five doses prevented the onset of clinical disease; the mean virus titer was decreased by 1-2 logs, and in some monkeys no detectable viremia was observed (TABLE 5). Untreated infected monkeys developed higher viremia with mild to severe clinical disease.[17]

Several of these compounds were very effective when administered after injection of the lethal virus challenge (TABLE 7). In most of the infections the treatment was effective when it was administered not later than 24 h postchallenge because 3-4 days after infection a large percentage of the infected mice died, and 5-7 days later all of them succumbed to the diseases. Of all the substances, HuRINF4-αB/D was the most efficacious against viruses from three arbovirus families; however, 50-100 higher units of INF were required compared with the prophylactic therapy (TABLE 5). Most of the substances were very effective against RVFV infection, which caused systemic infections in the early stage of the disease lasting for 3-5 days. Encephalitic infection is caused in about 20% of the mice in later stages of the infection. Once the virus enters and proliferates in the central nervous system (CNS), the immunomodulators have no effect because only very small amounts, if any, penetrate the blood-brain barrier. Flaviviruses probably reach the CNS more slowly when compared with alphaviruses, which may explain their better response to these substances. Alphaviruses appear to be the least sensitive to these substances, but even these viruses respond fairly well to delayed start of the therapy. Mice infected with VEE were completely protected by HuRINF-αB/D on delayed administration, which suggests that this biological might penetrate the blood- brain barrier. In any case, INF-α is the treatment of choice for VEE, and most likely for other alphaviruses as well. Four of the immunomodulators that were employed to treat pneumonia or encephalitis caused by herpes simplex viruses yielded a very high survival rate. This is reminiscent of the prophylactic treatment;

TABLE 7. Therapeutic Efficacy of Immunomodulators against Arbovirus Infections in Rodents and Nonhuman Primates

	Mouse									Monkey	
	Bunyaviridae			Flavivirus	Alphavirus		Miscellaneous		Guinea Pig	Bunyaviridae	Flavivirus
Compound (Source)	Rift Valley Fever	Punta Toro	Carapuru	*Banzi*	Semliki F.	Venezuelan Equine Enceph.	Herpes Simplex Pneumonitis	Herpes Simplex Enceph.	Arena Virus: Pichinde	Rift Valley Fever	Yellow Fever
Poly (ICLC) (NIAD, NIH)	++++	++++		++++	++	++	++++	++++	+		+++
Poly(ICDX) (Roswell Park, USAMRIID)	++++										
Ampligen (Johns Hopkins U.)	++	++++	+	+++	++	++	++++		0		
CL-246, 783 (Lederle)	++++	++++	+	++	+++	++	++	++++	0		
AVS-1018 (Riker-3M)	++++			++++		+			0		
Human recombinant INF-alpha (Ciba-Geigy, Hoffman LaRoche)	++++	++++	++	++++	+++	++++		++++		+++*	++++*
Mouse recombinant INF-gamma (Genentech)			0	+++	+	+					
ABPP (Upjohn)	+	++++		++		++					
AIPP (Upjohn)	0	0				0					

Key to symbols: 0 indicates not active (no survivors); +, marginal efficacy (up to 25% survivors); ++, moderate efficacy (26-50% survivors); +++, marked efficacy (51-75% survivors); ++++, high efficacy (76-100%); +++*, moderate reduction of circulating virus titer; ++++*, complete reduction of circulating virus titer.

when these substances were administered after infection with Pichinde virus representing Arenaviridae, no therapeutic benefit was evident. A moderate therapeutic response was evoked with ribavirin in Pichinde virus-infected guinea pigs when treatment was continued daily or every other day for a few days past the death of the untreated controls (TABLE 8). This moderate, but consistently repeatable, efficacy of ribavirin was considerably enhanced by the combined administration of ribavirin and poly(-ICLC), even though poly(ICLC) had no therapeutic efficacy in this particular study (TABLE 8). Occasionally, this substance resulted in a very marginal efficacy in other studies.

As already shown in TABLE 5, poly(ICLC) was also an effective therapeutic regimen when administered to rhesus monkeys not later than 8 h postinfection with yellow fever virus. When the treatment commenced 24 h postinfection, no therapeutic advantage was seen.[15] In African green monkeys, 5×10^5 IU/kg HuRINF-α (Hoffman LaRoche, Nutley, NJ) administered one day postinfection with the DakH strain of yellow fever virus and repeated daily for six more days, completely eliminated 6 logs of circulating virus titers (FIG. 4). Marked antiviral activity was also indicated by delayed and low neutralizing antibody response in this host as compared with the untreated controls; this was caused by the absence of antigenic mass required for stimulation of antibody production. Absence of infection was also indicated by normal levels of liver function and heart muscle enzymes in the treated host, while these enzyme levels were elevated in the untreated controls (not shown on the figure). Only a very low percent of the African green monkeys died of yellow fever virus infection; therefore, meaningful mortality data cannot be concluded from the numbers included in the study.

Although these substances stimulated a variety of biological functions, induction of INF is common to all of them, or exogenous INF itself is the inducer. Thus, it appears that INF has a central role in evoking the antiviral defenses in the host. Based on our data and data published by others,[18,19] a "two-armed" pathway appears to provide the antiviral mechanism of action of these immunomodulators (FIG. 5). Via the upper arm, an immunomodulator stimulates INF release by macrophages, T lymphocytes, and fibroblasts. The INF in turn activates 2'-5' AS and related enzymes, which inhibit viral proliferation. Via the lower arm, INF stimulates the specific and nonspecific cellular or humoral immune reactivity. Consequently, abrogation of INF by antiserum would eliminate both the upper and the lower arm of the antiviral mechanisms unless the upper arm of the response were reinduced. This is the case with poly(ICLC) or poly(ICLDx), which are double-stranded RNAs and as such are able to induce 2'-5' AS without prior induction of INF. Therefore, the antiviral activity of poly(ICLC) cannot be abrogated by anti-INF serum. QA (AVS 1018) and AH (CL246738) are not double-stranded RNAs; consequently, their antiviral activity was abrogated by administration of anti-INF serum. In the lower arm of the response, INF initiates the cytokine cascade via macrophage activation, and at the end of the activation process, both the viral-specific humoral and cellular immune response (B and T cells, respectively), and the nonspecific antiviral response exerted by macrophages, NK, and polymorphonuclear cells contribute to the inhibition of the infectious agent.

CONCLUSIONS

A large and economically significant number of domestic animals is affected by infections caused by arboviruses. Control of these infections by combined means of surveillance, immunization, and vector control were effective in preventing or in con-

TABLE 8. Treatment of Pichinde Virus[a] Infection in Guinea Pigs with Ribavirin, Poly(ICLC) or in Combination

Experiment	Compound	Route	Dose	Schedule (days)	Survivors (%)	MST[b] (days)
1	Ribavirin	i.p.	30 mg/kg	1-10, 12, 14, 16, 19, 20, 22	37	23.5
	Placebo	i.p.	0.3 mL	1-10, 12, 14, 16, 19, 20, 22	0	16.5
2	Ribavirin	i.p.	25 mg/kg	1-10, 12, 14, 16, 18, 20, 22	57	> 70.0
	Placebo	i.p.	0.3 mL	1-10, 12, 14, 16, 18, 20, 22	0	18.5
3	Ribavirin	i.p.	30 mg/kg	0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20	40	26.5
	Poly(ICLC)	i.p.	1 mg/kg	0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20	0	18.5
	Ribavirin + Poly(ICLC)	i.n.[c] + i.p.	30 mg/kg + 1 mg/kg	0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20	80	> 36.0
	Placebo	i.n.	0.1 mL	0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20	10	15.0

[a] Lethal virus challenge of 10^3 PFU was injected s.c. on day 0.
[b] MST, median survival time.
[c] i.n., intranasal.

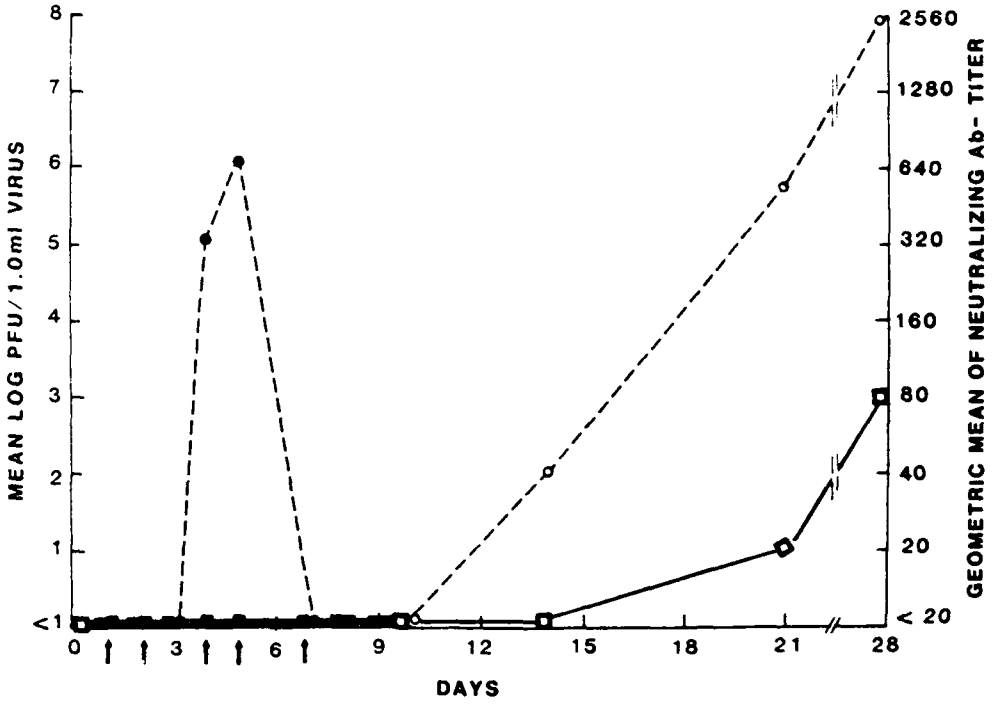

FIGURE 4. *Yellow fever virus and neutralizing antibody titer in the serum of cynomolgus monkeys treated with human leukocyte recombinant interferon-alpha (HuRINF-α).* ■——■ *Monkeys (n = 4) were treated s.c. with 5 × 10^5 International Units of HuRINF-α daily from day 1 to day 7, and were challenged s.c. on day 0 with 500 PFU DakH strain of yellow fever virus.* ●- - -● *Monkeys (n = 4) were treated s.c. with placebo daily from day 1 to day 7, and were challenged s.c. on day 0 with 500 PFU DakH strain of yellow fever virus.* □——□ *Neutralizing antibody titer in the serum of HuRINF-α-treated monkeys.* ○- - -○ *Neutralizing antibody titer in the serum of placebo-treated monkeys.*

taining outbreaks of arboviral infections in several countries of the Caribbean Basin. Nevertheless, several endemic and epidemic outbreaks occurred in the region, which underscores the necessity for additional controlling measures.

Immunomodulators are eminently suitable to provide protection when other means of control fail. Several immunomodulators can protect against viruses belonging to three of the four arbovirus families. Viruses belonging to the fourth family respond well to treatment with ribavirin. Because it is not practical to identify the viral infection to be treated, combined administration of an immunomodulator and ribavirin would cover all arbovirus infections. During an epidemic, domestic animals in large geographical areas are eliminated to prevent the spread of the disease, even though the majority of them is not infected, as indicated by the absence of antibodies in retrospective studies. The use of immunomodulators in domestic animals would also invaluably contribute to the preclinical database required for human clinical safety and efficacy studies. Most of the immunomodulators do not require large financial expenses, and 10-14 days of treatment is usually sufficient during epidemics. Repeated administration of these substances can be resolved by delivery of the substance in microencapsulated form. A single administration of a biodegradable, biocompatible microsphere that can release the encapsulated immunomodulator with a constant rate for a predetermined period of time is sufficient to prevent lethal RVFV infection.[20]

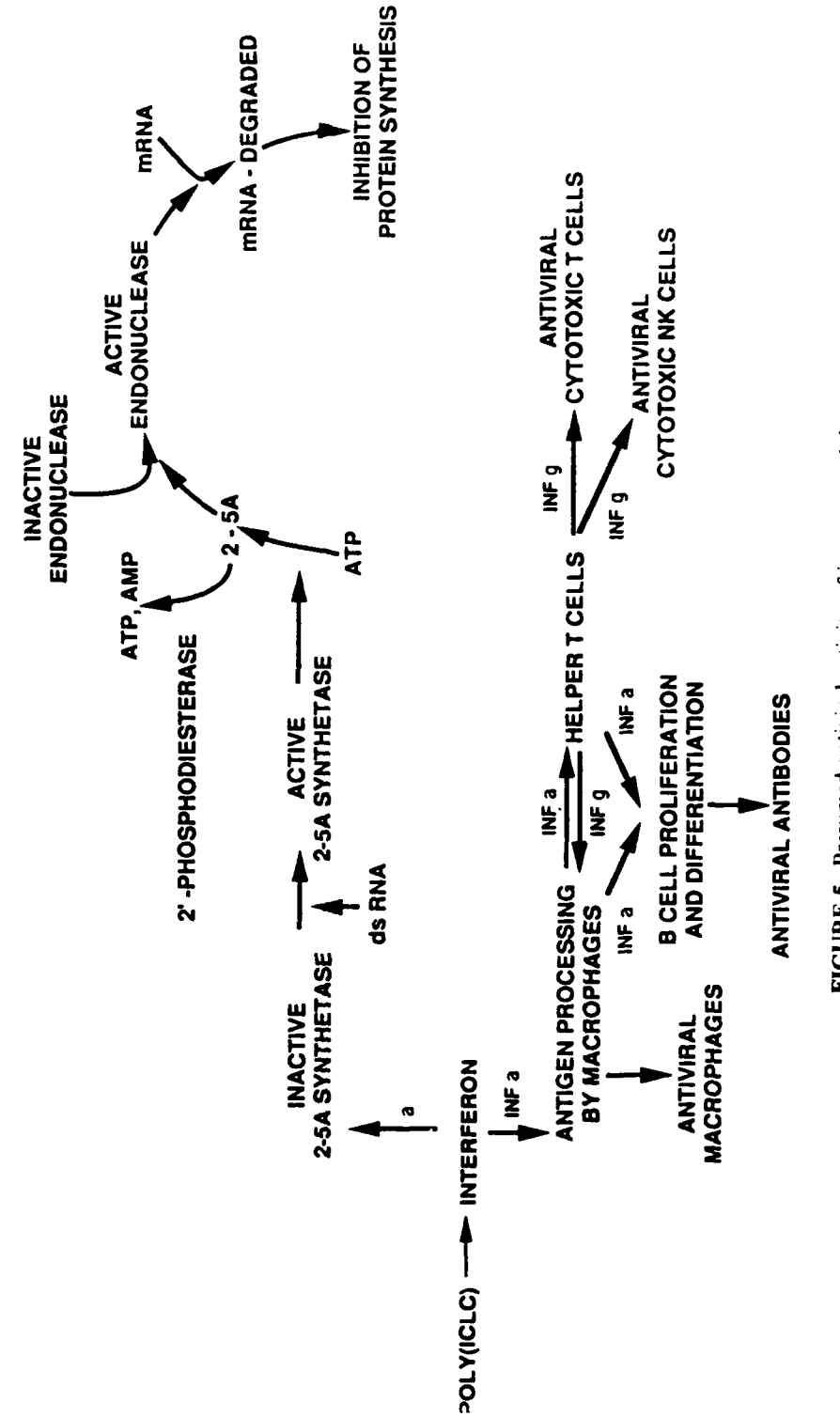

FIGURE 5. *Presumed antiviral activity of immunomodulators.*

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